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Insecticidal effect of spinosad dust, in combination with diatomaceous earth, against two stored-grain beetle species

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ABSTRACT

Laboratory bioassays were carried out to determine the efficacy of spinosad applied alone or combined with the diatomaceous earth (DE) SilicoSec against adult rice weevils, *Sitophilus oryzae* and confused flour beetles, *Tribolium confusum*. Efficacy was assessed on wheat and maize at three dosages of spinosad dust formulation (corresponding to 0.0625, 0.1875 and 0.625 ppm of active ingredient [AI] for *S. oryzae* and to 0.1875, 0.625 and 1.25 ppm of AI for *T. confusum*), alone or combined with SilicoSec at 150 ppm for *S. oryzae* and 250 ppm for *T. confusum*. The mortality of *S. oryzae* exposed for 14 d on wheat treated with spinosad ranged between 83% and 100%. Conversely, the mortality of *S. oryzae* on maize treated with DE or on maize treated with lower doses of spinosad dust did not exceed 19% and was only 59% on maize with the highest spinosad dust treatment. Generally, the presence of SilicoSec combined with spinosad did not significantly increase *S. oryzae* mortality compared with spinosad alone. For *T. confusum*, mortality on both commodities was lower than for *S. oryzae*. After 14 d of exposure on wheat, mortality was 14% at the highest dose of spinosad, but increased to 33% in the presence of DE. Similar results were also obtained for *T. confusum* exposed on treated maize, which indicated a joint action between spinosad and DE. In the case of *S. oryzae*, the inclusion of DE reduced progeny production in comparison with spinosad alone. Progeny production of *T. confusum* was relatively low in all treatments, compared to progeny production of *S. oryzae*. The results of the study show the potential of combination treatments of spinosad dust and DE, but efficacy varies with the target insect species and commodity.

1. Introduction

Concerns regarding the use of conventional neurotoxic insecticides as grain protectants, including difficulties in re-registration of those insecticides in some countries, concerns regarding mammalian toxicity, and issues with residues on food, have led researchers to the evaluation of new reduced-risk insecticides to control stored-product insects. Spinosad is a broad-spectrum insecticide based on metabolites of *Saccharopolyspora spinosa* Mertz and Yao (Bacteria: Actinobacteridae), and is a promising alternative to neurotoxic insecticides because it is based on a natural product, has low mammalian toxicity, and is effective against a wide range of stored-product insects (Thompson et al., 1997; Subramanyam et al., 2003). Spinosad is effective as a protectant of stored grains (Fang et al., 2002; Subramanyam et al., 2003; Subramanyam, 2006) and as a residual application to flooring surfaces (Toews et al., 2003). Most of the published studies involved the use of spinosad as a liquid formulation, but it is also available as a dust. The use of spinosad dust formulation may be more advantageous than a liquid formulation in some cases because the dust can be removed from the treated grain before it is milled. Moreover, there is evidence that liquid spinosad is not equally effective among different grains (Fang et al., 2002; Subramanyam, 2006).

One other promising alternative to conventional neurotoxins is the use of diatomaceous earth (DE), which is comprised of fossils of phytoplanktons (diatoms) and acts through absorption of lipids from the insect cuticle and partially through abrasion (Korunic, 1998; Subramanyam and Roesli, 2000). Commercial DE formulations have low mammalian toxicity, and can be removed from the grain during the milling process (Korunic et al., 1996; Korunic, 1998). Many DE formulations are now commercially available, and are generally effective against stored-grain beetles (Subramanyam and Roesli, 2000; Fields and Korunic, 2000; Arthur, 2003; Athanassiou et al., 2004). However, even when used at the label rates, DE has adverse effects on physical properties of grains,

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particularly bulk density and flow rate (Korunic et al., 1998). Moreover, DEs are not equally effective on all grain commodities or all beetle species, which means that variable application rates are needed depending on target species and the particular grain (Athanassiou et al., 2003; Athanassiou and Kavallieratos, 2005; Kavallieratos et al., 2005). One possible solution to this problem is the use of enhanced DE, which contains a small amount of insecticide with low mammalian toxicity together with the DE (Athanassiou et al., 2004, 2006). For example, Athanassiou et al. (2006) reported that DEBBM, which is a combination of DE with the plant extract bitterbarkomycin, was very effective at concentrations of 150 ppm or less. A similar strategy using a combination of DE with spinosad dust may also be an effective means of control of stored-grain insects. Therefore, the objectives of this investigation were to: (1) evaluate different combination treatments of DE and spinosad on wheat and maize for effectiveness against a primary pest beetle species, the rice weevil *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), and a secondary pest, the confused flour beetle *Tribolium confusum* du Val (Coleoptera: Tenebrionidae); and (2) assess progeny production after exposure of parental adults on the treated grains.

2. Materials and methods

2.1. Test insects

The test insects used in our study were 1–4-week-old adult *S. oryzae* and *T. confusum*. The former species was reared on whole wheat and the latter on wheat flour plus brewer's yeast (5% by weight). Both species were reared at $27\pm1\,^{\circ}$C, 60 ± 5% relative humidity (r.h.) in continual darkness.

2.2. Commodities

Untreated, clean and infestation-free hard wheat (var. Mexa) and maize (var. Dias), with dockage content <1%, were used in the tests. The moisture content of the grains, as determined by a Dickey–John moisture meter (Dickey–John multigrain CAC-II, Dickey–John Co., Auburn, IL, USA), ranged between 10.9% and 11.5%. Before the beginning of the experiments, the grain quantities were left at ambient conditions (see below) for 7 d, to equilibrate with the r.h. level.

2.3. Formulations

The spinosad dust (dry) formulation used in the experiments contained 0.125% active ingredient (AI) (Premier Shukuroglou Ltd., Nicosia, Cyprus). The DE formulation used was SilicoSec (Biofa GmbH, Germany). SilicoSec is a DE of freshwater origin that contains approx. 92% SiO_2 (Athanassiou et al., 2004).

2.4. Grain treatment

Spinosad and SilicoSec were applied at different dose rates for *S. oryzae* and *T. confusum*, as preliminary tests indicated that these two species were not equally susceptible to these two substances. Hence, the spinosad dose rates tested for *S. oryzae* were 50, 150 and 500 ppm of the formulation, corresponding to 0.0625, 0.1875 and 0.625 ppm of AI. For *T. confusum* the dose rates were 150, 500 and 1000 ppm, corresponding to 0.1875, 0.625 and 1.25 ppm of AI. The SilicoSec dose rates selected were 150 and 250 ppm for *S. oryzae* and *T. confusum*, respectively. For each species, there were seven treatments: three doses of spinosad, three doses of spinosad with DE, and DE alone (Table 1). For each specific treatment combination, for each grain, lots of 1 kg were prepared and placed in cylindrical jars, and the appropriate amount of dust product (spinosad and/or SilicoSec) was added to the jars. The jars were then shaken manually for 10 min to achieve equal distribution of the dust in the entire grain mass. For each grain, there was an additional untreated lot which was used as the control.

2.5. Bioassays

Three samples of 30 g each were taken from each lot, and placed in a small cylindrical glass vial. Thirty mixed-sex adults of *S. oryzae* were introduced into each vial. The same procedure was followed for *T. confusum*. The vials were then placed in incubators set at 25 °C, 55% r.h. and continual darkness. Parental adults were counted after 7 and 14 d, dead adults were tabulated and removed from the vials. The test was repeated three times (3×3 vials) by using new lots of grains each time. After the 14-d mortality count, all parental insects were removed and the vials returned to the incubators and held at the same conditions for an additional 65 d. After this interval, the vials were opened and the grains were examined for adult progeny emergence.

2.6. Data analysis

Control mortality, which was generally low and in most cases did not exceed 5%, was corrected using Abbott's formula (Abbott, 1925). Before the analysis, mortality and progeny production data were arcsine and logarithmically [ln(x+1)] transformed, respectively. Since the same vials were examined for mortality at 7 and 14 d, the mortality data were analyzed by using a repeated measures ANOVA with exposure as the repeated measures variable, by using JPM IN 5.1 software (Sall et al., 2001). For each species, exposure interval and commodity, the mortality data were submitted to ANOVA to determine differences among treatments. The same procedure was also followed to analyze data for progeny production. A preliminary ANOVA indicated that the number of progeny in the untreated vials was significantly higher than that in the vials containing the treated grains, therefore the untreated controls were eliminated to obtain significances among treatments.

Table 1
Concentrations of spinosad (actual amount of active ingredient [AI]) and diatomaceous earth (DE, SilicoSec) used for each species

Species	Spinosad alone (ppm AI)	SilicoSec alone (ppm)	Combination (spinosad+SilicoSec) (ppm)
S. oryzae	50 ppm (0.0625)	150	50+150
	150 ppm (0.1875)		150+150
	500 ppm (0.625)		500+150
T. confusum	150 ppm (0.1875)	250	150+250
	500 ppm (0.625)		500+250
	1000 ppm (1.25)		1000+250

Means were separated by the Tukey–Kramer Honestly Significant Difference (HSD) test at $P<0.05$ (Sokal and Rohlf, 1995).

3. Results

3.1. Parental mortality

All main effects and associated interactions were significant for mortality of parental adults (Table 2). After 7 d of exposure, mortality of *S. oryzae* adults exposed on wheat treated with spinosad increased with increasing concentration, regardless of the presence of SilicoSec. At 7 d exposure, significant differences were noted in mortality levels among treatments (Fig. 1A), with greater mortality on wheat treated with the highest concentration of spinosad. The presence of DE did not cause a significant increase in mortality, except at the lowest spinosad dose. However, even in this case mortality was similar or even lower than the mortality caused by SilicoSec alone. After 14 d of exposure, mortality increased numerically, but in most cases there were no significant differences among treatments (Fig. 1B). All weevils exposed on wheat treated with the highest spinosad dose combined with SilicoSec were dead after 14 d.

Table 2
Repeated measures ANOVA parameters for the species tested

	df	*S. oryzae*		*T. confusum*	
		F	*P*	*F*	*P*
Source between variables					
All between	13	17.4	<0.001	14.8	<0.001
Intercept	1	33.5	<0.001	281.9	<0.001
Commodity	1	1602.5	<0.001	1.3	0.2574
Treatment	6	49.7	<0.001	31.4	<0.001
Commodity × treatment	6	8.5	<0.001	0.5	0.7769
Source within variables					
Within interactions	13	16.0	<0.001	16.1	<0.001
Exposure	1	276.4	<0.001	291.2	<0.001
Exposure × commodity	1	43.5	<0.001	6.4	0.0123
Exposure × treatment	6	6.1	<0.001	33.2	<0.001
Exposure × commodity × treatment	6	21.3	<0.001	0.5	0.7756

At the 7-d exposure interval, mortality of adult *S. oryzae* was notably lower on maize compared with the respective values on wheat (Fig. 1C). The number of dead adults was greater on maize treated with 1000 ppm of spinosad, where mortality was 40% and 33% with or without DE, respectively, compared with the other concentrations, where mortality did not exceed 12%. Similar trends were also noted after 14 d of exposure, where mortality reached 59% and 51% at 1000 ppm of spinosad with or without SilicoSec, respectively (Fig. 1D). Mortality in the other combinations was <19%.

When *T. confusum* adults were exposed for 7 d, mixed significance was obtained for main effects and interactions (Table 2). Mortality of *T. confusum* adults was lower in comparison with the respective mortality levels for *S. oryzae*, on both wheat and maize (Fig. 2). After 7 d of exposure on wheat, mortality did not exceed

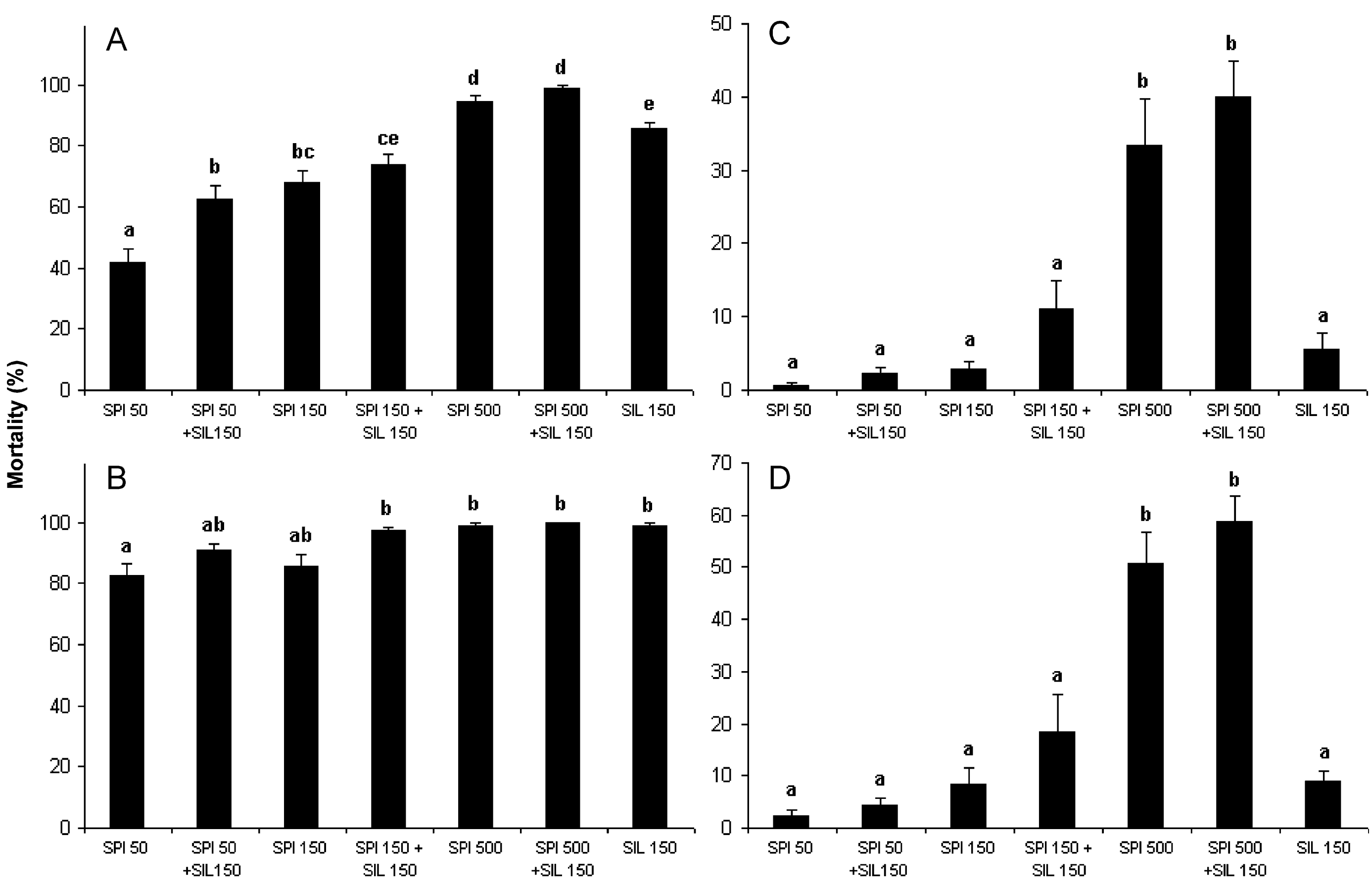

Fig. 1. Mean (%) mortality (±SE) of *S. oryzae* adults exposed for 7 or 14 d on wheat or maize treated with spinosad (SPI) and SilicoSec (SIL) at various combinations (A, B, wheat after 7 and 14 d, respectively, C, D, maize after 7 and 14 d, respectively; within each diagram means accompanied by the same letter are not significantly different; for 7 d on wheat $F=34.6$, 14 d on wheat $F=9.6$, 7 d on maize $F=20.1$, 14 d on maize $F=7.6$, in all cases df = 6.56, $P<0.01$; HSD test at 0.05).

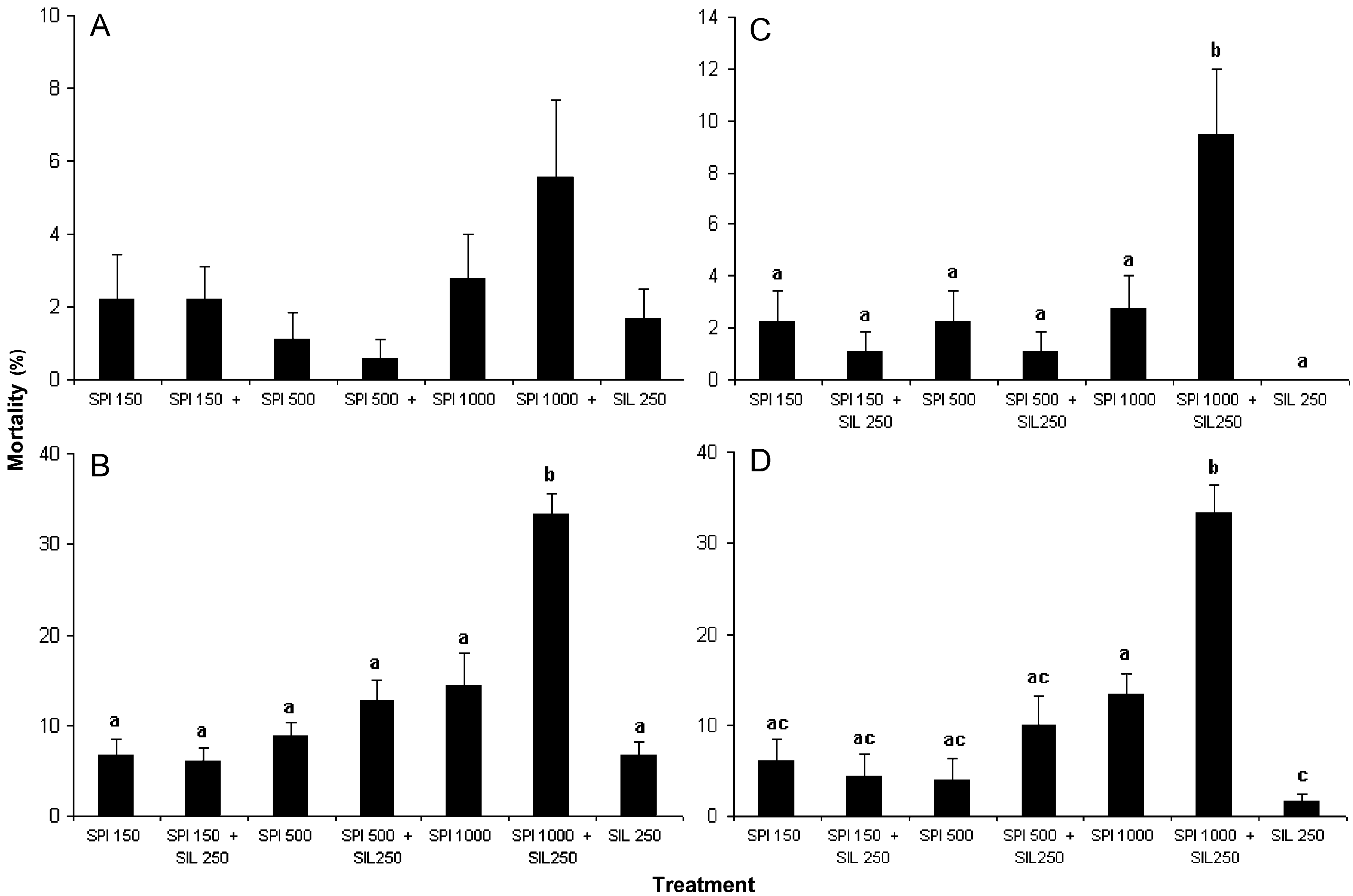

Fig. 2. Mean (%) mortality (±SE) of *T. confusum* adults exposed for 7 or 14 d on wheat or maize treated with spinosad (SPI) and SilicoSec (SIL) at various combinations (A, B, wheat after 7 and 14 d, respectively, C, D, maize after 7 and 14 d, respectively; within each diagram means accompanied by the same letter are not significantly different; where no letters exist, no significant differences were noted; for 7 d on wheat $F = 1.9$, $P = 0.10$, 14 d on wheat $F = 19.5$, $P<0.01$, 7 d on maize $F = 5.6$, $P<0.01$, 14 d on maize $F = 20.2$, $P<0.01$, in all cases df = 6.56; HSD test at 0.05).

6% and there were no significant differences among treatments (Fig. 2A). After 14 d, there were more dead adults in wheat treated with the highest spinosad rate combined with SilicoSec in comparison with the other treatments (Fig. 2B). However, mortality was still only 33%.

When *T. castaneum* adults were exposed on maize, significantly more adults were dead after 7 d of exposure to 1000 ppm spinosad combined with SilicoSec compared to the other treatments, but mortality was still <10% (Fig. 2C). In addition, all adults survived when exposed on maize treated with SilicoSec alone. At the 14-d exposure, the presence of SilicoSec significantly increased the efficacy of the highest dose rate of spinosad, in comparison with the other treatments (Fig. 2D). Also, mortality on maize treated with SilicoSec alone was significantly lower than mortality on 1000 ppm of spinosad.

3.2. *Progeny production*

Mean progeny production in the controls for *S. oryzae* was 126.9±21.5 weevils/vial. In vials containing either wheat or maize, an increasing concentration of spinosad significantly decreased emergence of *S. oryzae*, with or without DE (Fig. 3A and B). Also, the presence of DE alone generally did not lead to a significant decrease in progeny production. More progeny were produced on maize than on wheat, while for both commodities, DE significantly decreased progeny at the lowest spinosad dose. For *T. confusum*, mean progeny production in the untreated vials was 20.4±7.1 adults/vial; progeny production of *T. castaneum* in vials containing treated grains did not exceed 0.3 adults/vials (Fig. 3C and D).

4. Discussion

Our study shows that the dry spinosad formulation can be used against *S. oryzae* and *T. confusum*, but several variables affect its efficacy, such as the exposure interval, the specific grain commodity, and the target pest species, Variable effects on different beetle species have also been noted in recent studies with spinosad dust. Mutambuki et al. (2003) found that concentrations of <1 ppm of spinosad dust were very effective against adults of the larger grain borer, *Prostephanus truncatus* (Horn), but less effective for the maize weevil, *Sitophilus zeamais* (Motschulsky). Getchell (2006) also reported that spinosad was more effective against the lesser grain borer, *Rhyzopertha dominica* (F.) than against *S. oryzae*, on grains treated with both dry and liquid formulations of spinosad. In studies with liquid spinosad alone, 1 ppm provided 100% mortality of *S. oryzae* adults in durum wheat after 7 d of exposure (Fang et al., 2002), but higher concentrations were needed to produce complete mortality of

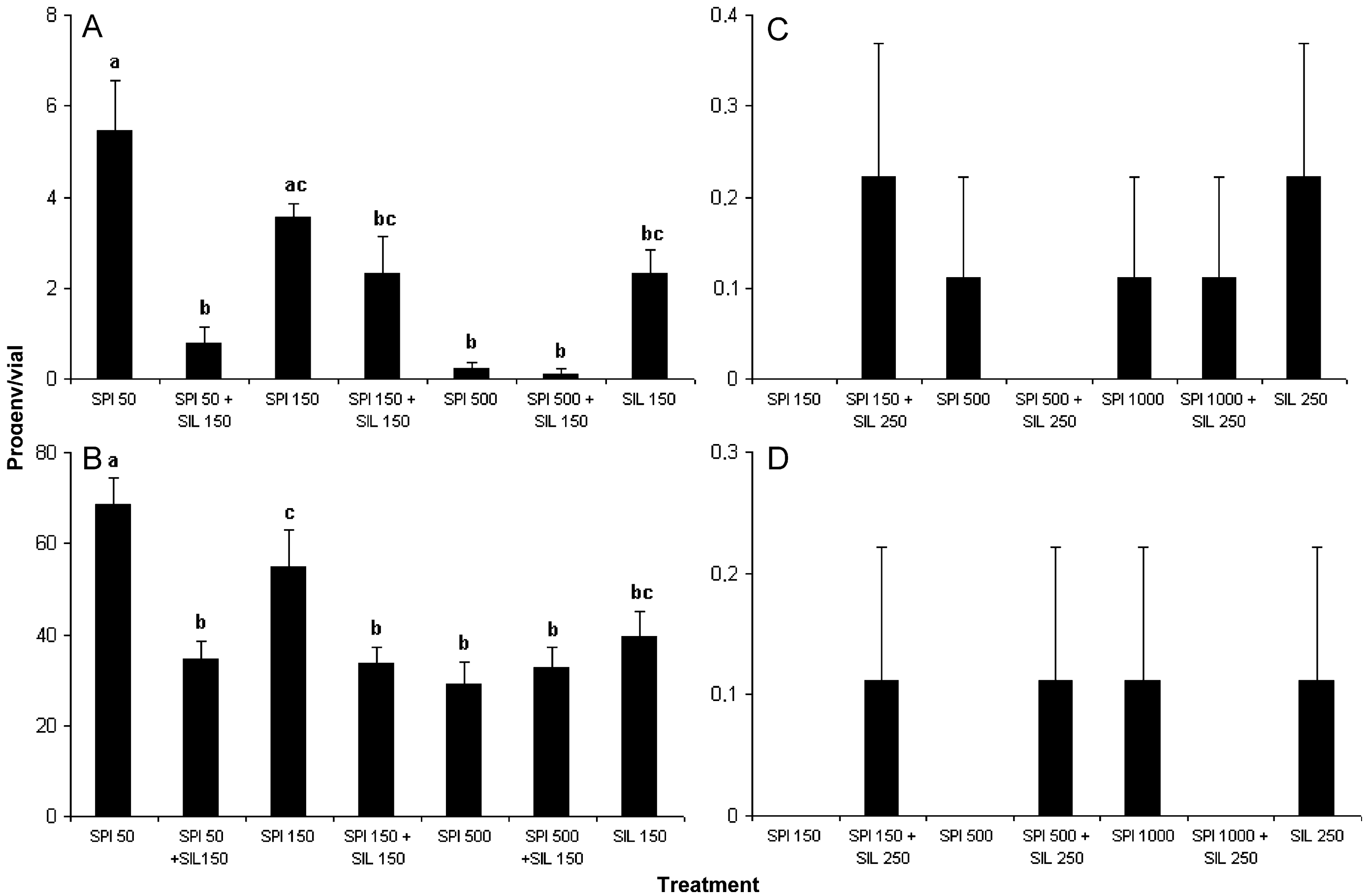

Fig. 3. Mean progeny production (number of individuals/vial ± SE) 65 d after the removal of the parental adults from wheat or maize treated with combinations of spinosad (SPI) and SilicoSec (SIL), for *S. oryzae* (A for wheat, B for maize) and *T. confusum* (C for wheat, D for maize) (within each diagram means accompanied by the same letter are not significantly different; where no letters exist, no significant differences were noted; for *S. oryzae* on wheat $F = 21.2$, $P < 0.01$, on maize $F = 7.6$, $P < 0.01$, for *T. confusum* on wheat $F = 0.7$, $P = 0.64$, on maize $F = 0.5$, $P = 0.81$, in all cases df = 6.56; HSD test at 0.05).

Tribolium spp. compared to *S. oryzae* (Liang et al., 2002; Subramanyam et al., 2003; Toews and Subramanyam, 2003; Huang et al., 2004).

The presence of SilicoSec increased spinosad efficacy against *S. oryzae* at the lowest concentrations, but the benefit of this increase could be considered negligible given that the efficacy of SilicoSec was comparable to that of spinosad at the higher concentrations. Previous studies document that SilicoSec is very effective against *S. oryzae* (Athanassiou et al., 2003, 2004, 2005). For instance, Athanassiou et al. (2003) reported that 125 ppm of SilicoSec provided 77% mortality of *S. oryzae* in barley after 14 d of exposure. The high SilicoSec efficacy recorded for *S. oryzae* could be attributed to the r.h. level of 55%, since the efficacy of DE decreases with increasing humidity (Korunic, 1998; Fields and Korunic, 2000; Arthur, 2003; Vayias and Athanassiou, 2004). Hence, any potential additive effect may have been concealed by the high SilicoSec efficacy.

In contrast with *S. oryzae*, the combination treatments did show an additive effect on *T. confusum* adults. This could be attributed to the fact that the efficacy of SilicoSec, as well as of other DEs, is low against this species (Vayias and Athanassiou, 2004) and the closely related red flour beetle *Tribolium castaneum* (Herbst) (Fields and Korunic, 2000). Arthur (2000, 2003) and Athanassiou et al. (2004) found that *S. oryzae* adults are much more susceptible to DE than *Tribolium* adults after exposure to grains treated with the DEs Protect-It, SilicoSec, PyriSec and Insecto. In addition, *T. confusum* and *T. castaneum* are among the most tolerant stored-product beetle species to spinosad, since 1 ppm or higher of liquid spinosad is required to control these species (Subramanyam et al., 2003; Toews et al., 2003). In our study, even these dose rates were not sufficient to control *T. confusum* adults, indicating that for this species, spinosad dust in less effective than liquid spinosad. Thus, *T. confusum* and *T. castaneum* appear to be good test species for further examination of additive effects of spinosad–DE combinations. In our study, spinosad efficacy was 2.5–4 times higher with SilicoSec than with spinosad alone, for some specific doses of the combination. In addition, SilicoSec efficacy, at 250 ppm, was low, therefore a combination treatment with spinosad would be more appropriate for *T. confusum* or *T. castaneum* than for other stored-grain beetles. Spinosad acts through both contact, ingestion, and through the nervous system, while DE acts through absorption at the insect cuticle. Adults stressed through contact with the DE-treated substrate could be more vulnerable to spinosad because desiccation caused by DEs may indirectly increase the metabolic stress, thereby increasing spinosad activity.

Differences in toxicity depending on commodity were also apparent in our test. With many DE formulations, including SilicoSec, there are differences in toxicity when applied to different grains. Athanassiou et al. (2003) reported that mortality of *S. oryzae* adults in rice treated with SilicoSec was greater than in maize, regardless of the dose rate. Vayias et al. (2006) noted similar differences between maize and wheat for *T. confusum*. Toews et al. (2003) found significant differences in mortality levels of *T. confusum* adults among different surfaces treated with spinosad. However, Getchell (2006) found that both liquid and dust formulations performed equally well on wheat, maize and sorghum, against *S. oryzae* and *R. dominica*.

The degree of adherence, retention and distribution of spinosad dust on the surface of different grain kernels and the potential interaction of spinosad with broken grain and dockage, and with the morphological characteristics of the external kernel parts, have not been examined in published research studies. In trials with the DEs SilicoSec, PyriSec and Insecto, Athanassiou and Kavallieratos (2005) and Kavallieratos et al. (2005) found varying degrees of adherence of DE particles among eight different grain commodities. In these commodities, the lowest retention rate of 11% was found on maize, while the retention rate on wheat and rice was $>80\%$. This may partially explain the difference in efficacy of dusts not only for DE. On the other hand, spinosad may interact with specific substances that occur at the kernel surface, which may partially explain differences in efficacy among grains. Fang et al. (2002) found considerable differences in spinosad efficacy against several stored-grain beetle species between different classes of wheat, but there was no correlation between efficacy and kernel diameter, hardness, fiber, weight or protein. Additional experimentation is required to examine the factors that affect these differences among grains, with emphasis in physical differences of dust particles–kernel interactions.

Suppression of the subsequent generations is one of the basic characteristics of a successful grain protectant (Arthur, 1996). Spinosad is capable of giving long-term protection without a loss in efficacy (Fang et al., 2002; Maier et al., 2006). However, there are some indications of reproduction even with high parental mortality (Fang et al., 2002). In our tests, progeny production was higher for *S. oryzae* than for *T. confusum*, probably because *S. oryzae* is an internal feeder while *T. confusum* is an external feeder. Hence, larvae of *S. oryzae* are protected from residues on the kernels, as occurs with DE (Arthur and Throne, 2003). In contrast, larvae of *T. confusum* are in direct contact with either spinosad or DE particles; thus, even if parental mortality of *T. confusum* is low, progeny production will be limited and the population could be gradually eliminated. Nevertheless, *Tribolium* spp. cannot develop very easily in sound kernels. In the present work, whole kernels were used; hence, progeny production is likely to be increased with increased broken kernel or dockage content. Moreover, the presence of broken commodity might have reduced parental mortality.

In conclusion, the present study documents that the combination of spinosad dust and DEs has potential use for control of both *S. oryzae* and *T. confusum*, since there is evidence for an additive effect. However, the differential susceptibility of these two pests and other stored-grain beetles, may require different rates for different commodities. Also, a higher concentration of AI may be needed for spinosad dust formulations, in order to lower application rates of the dust formulation.

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